

Mechanisms of bamboo witches' broom symptom development caused by endophytic/epiphytic fungi

Eiji Tanaka

Department of Environmental Science and Engineering; Ishikawa Prefectural University; Nonoichi-cho, Ishikawa Japan

Aciculosporium take causes continuous shoot growth but maintains normal leaf-arrangement and branching patterns in the host plant, which eventually resulting in witches' broom disease of bamboo. An in situ hybridization technique with a species-specific oligonucleotide probe was recently used to demonstrate that endophytic mycelia of *A. take* is predominantly distributed in the intercellular spaces of the shoot apical meristem of the host. Endophytic hyphae in meristematic tissues, which may produce auxin, are responsible for continuous primordium initiation within the shoot apex. Here I examine another bamboo witches' broom causal fungus, *Heteroepichloë sasae*. Both species are biotrophic and belong to family Clavicipitaceae: however, *H. sasae* does not cause continuous shoot growth. Histological study showed that *H. sasae* mycelia were distributed superficially, even on shoot apical meristems. These observations suggest that when their stromata develop, endophytic *A. take* destroys shoot apical meristem and epiphytic *H. sasae* chokes the shoot apex of the host. Stromata formation consequently causes lateral bud outgrowth because of release from apical dominance. This process repeats and eventually results in the witches' broom symptoms.

Causal Fungi of Bamboo Witches' Broom

Aciculosporium take (Ascomycota; Clavicipitaceae) is a causal agent of bamboo witches' broom disease in East Asia. Colonized shoots by *A. take* continue to

grow in an acropetal sequence with very thin stems and little leaves, although normal bamboo shoots cease to grow when three to five leaves develop (Fig. 1). The elongating shoot closely resembles a stolon or a vine but not super-elongation diseases such as bakanae disease. When the stroma is formed at the shoot apex, lateral buds grow out. Leaf arrangement and branching patterns are maintained even in colonized shoots. As with *Epichloë/Neotyphodium* endophytes, external fungal materials other than stromata are not observed on plant surfaces. The location of endophytic mycelia is probably involved in well-regulated symptoms. The author recently demonstrated that the endophytic mycelium of *A. take* was predominantly distributed in the intercellular spaces of shoot apical meristems of the host bamboo plant.¹ Endophytic hyphae were visualized by the in situ hybridization technique with a species-specific oligonucleotide probe.

Heteroepichloë sasae (Ascomycota; Clavicipitaceae) also causes witches' broom in small bamboo plants (e.g., *Sasa* spp.) in Japan and China.^{2,3} As with *A. take*, this fungus is included in the family Clavicipitaceae: their phylogenetic relationships were examined.⁴ Unlike *A. take*, *H. sasae* does not cause continuous shoot elongation. Its stroma encloses undeveloped leaves of the host and does not penetrate the leaf tissue.² Its habitat before stroma formation, its association with the host, and the mechanisms of symptom development have not been addressed thus far. In this addendum, a comparative study was performed between *A. take* and *H. sasae* to observe the development of bamboo witches' broom symptoms.

Key words: apical dominance, endophyte, epiphyte, histology, shoot development, witches' broom symptom

Submitted: 12/02/09

Accepted: 12/02/09

Previously published online:
www.landesbioscience.com/journals/psb/article/10834

Correspondence to: Eiji Tanaka;
Email: tanakae@ishikawa-pu.ac.jp

Addendum to: Tanaka E. Specific in situ visualization of the pathogenic endophytic fungus *Aciculosporium take*, the cause of witches' broom in bamboo. Appl Environ Microbiol 2009; 75:4829-34; PMID: 19465522; DOI:10.1128/AEM.00635-09.



Figure 1. Bamboo shoot (*Phyllostachys bambusoides*) colonized by *Aciculosporium take*. The colonized shoot continues to grow and finally generates stroma at the shoot apex: it has small leaves from the respective node, although normal shoots have three to five leaves. Bar, 10 cm.

Distribution of Mycelia in Host Plant

Cross sections of colonized shoots with stromata were observed to examine the distribution of mycelia (Fig. 2A and B). Sections were stained as mentioned in the legend for Figure 2. Stroma of *A. take* was formed on the host stem and was connected to the internal mycelia (Fig. 2A). Endophytic mycelia were observed in intercellular spaces of the stem tissue, and hyphae emerged between epidermal

cells to form stroma (Fig. 2C). Stroma of *H. sasae* enclosed undeveloped leaves and filled in the space between leaves (Fig. 2B). No mycelium was observed within leaf tissue, except for stomatal apertures (Fig. 2D). It is noted that *H. sasae* does not seem to intrude even from the stomata.

Longitudinal sections of the colonized shoot without stromata were observed to examine the association of fungi with shoot apex (Fig. 2E and F). Endophytic mycelia of *A. take* were observed in the intercellular

spaces of shoot apical meristem tissue (Fig. 2E), as shown previously.¹ In contrast, mycelia of *H. sasae* were observed on the surface of shoot apical meristem (Fig. 2F). These observations strongly suggest that *H. sasae* is entirely an epiphyte. The mycelial distributions of *H. sasae* are similar to that of *Myriogenosora* species that is clavicipitaceous fungi and causes dwarfing of host.^{5,6} Vegetative mycelia of both species may enlarge to develop stromata.

Mechanisms of Bamboo Witches' Broom Symptom Development

The developmental mechanisms responsible for continuous shoot elongation by *A. take* were discussed previously.¹ In brief, colonized shoots may not be able to produce a sufficient amount of endogenous free IAA to expand leaves and stems, whereas endophytic *A. take* hyphae within the apical meristem may continue to produce exogenous free IAA for inducing primordium initiation and maintaining apical dominance.^{1,7} This histological study suggests that *A. take* stroma formation destroys the shoot apical meristem, as a result of which endophytic hyphae pass through the epidermis to form stroma. On the other hand, witches' broom disease caused by *H. sasae* may develop as follows. Superficial mycelia of *H. sasae* thicken and become stroma, although it is unclear how nutrients are obtained from the host. The stroma prevents the leaf blade from expanding and eventually chokes the shoot. These observations indicate that both biotrophic fungi finally terminate the function of the host shoot apex when they form stromata. Consequently, lateral buds start growing because of release from apical dominance. Repetition of this sequence may result in symptoms of witches' broom disease.

Endophytic hyphae of *A. take* appear to regulate shoot development. However, other endophytic clavicipitaceous fungi have an insignificant effect on shoot development. For example, essentially symptomless *Neotyphodium* endophytes growing entirely in the intercellular spaces also inhabit the shoot apical meristem of the host.⁸ The study of these host-fungus relationships may provide insight into the shoot development process.

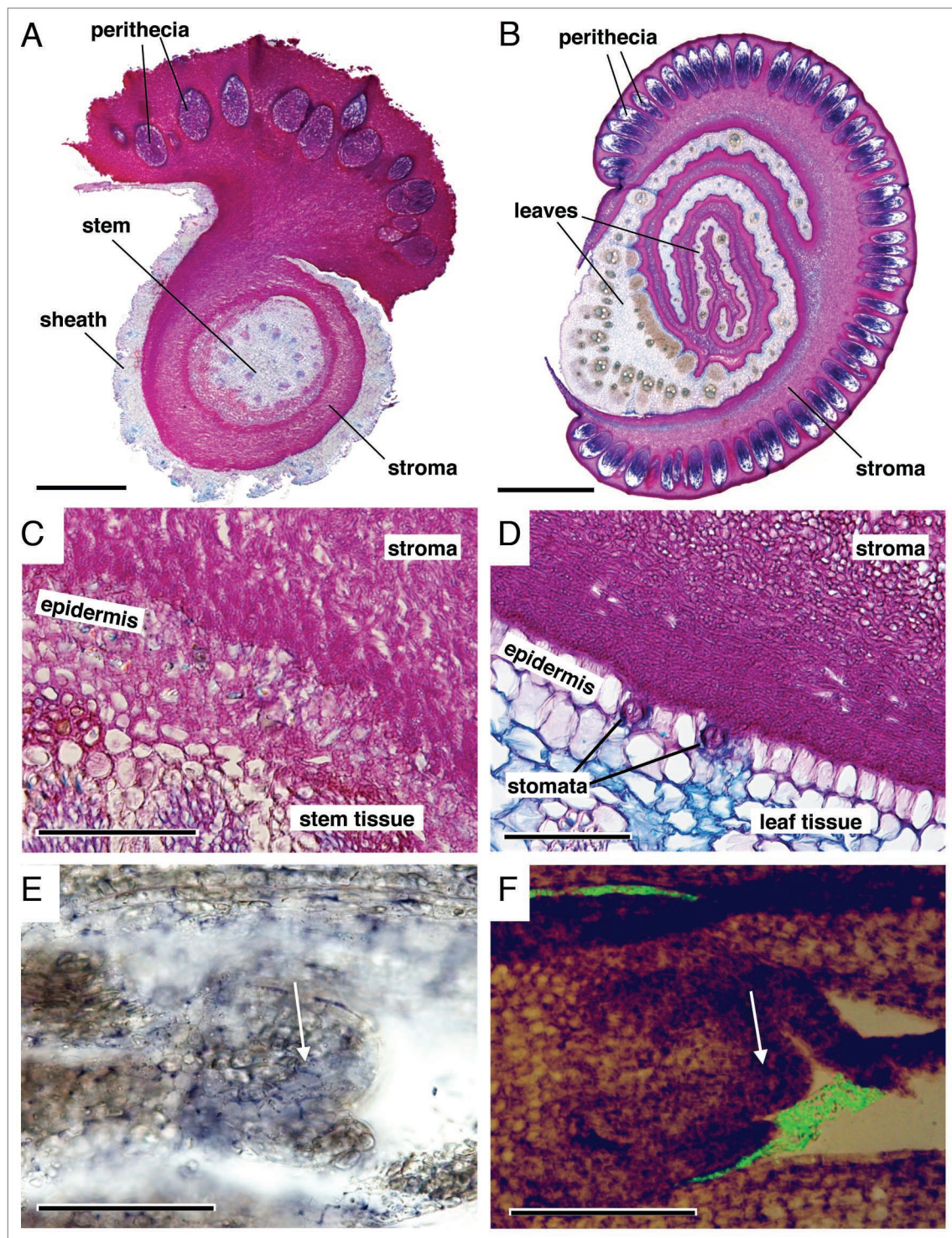


Figure 2. For figure legend, see page 418.

Figure 2. Comparisons between bamboo shoot tissue colonized by *Aciculosporium take* and *Heteroepichloë sasae*. Both species cause bamboo witches' broom disease. Samples were fixed, embedded in paraffin, and sectioned as shown previously.¹ All colonized bamboos were collected at Kanazawa-shi, Ishikawa, Japan (36°30'25"N, 136°37'59"E). (A–D) Sections stained by Alcian blue (pH 2.5) and periodic acid Schiff reaction methods.⁹ The magenta color indicates fungal materials. Perithecia with asci were stained blue-purple. (A and B) Several images were combined into one large image because the field of view obtained by a microscope was smaller than the sample dimensions. (A) Cross section of *A. take* stroma with perithecia. Bar, 500 μ m. (B) Cross section of *H. sasae* stroma with perithecia. Bar, 1 mm. (C) Interface between *A. take* stroma and the host plant tissue. Endophytic mycelia were observed in the intercellular spaces of plant tissue. Bar, 100 μ m. (D) Interface between *H. sasae* stroma and host plant tissue. Mycelia were observed superficially on the plant surface, except for stomata. Bar, 100 μ m. (E) Longitudinal section of an *A. take*-colonized shoot of *P. bambusoides*. The blue color indicates fungal mycelia visualized by in situ hybridization with a species-specific oligonucleotide probe.¹ Endophytic hyphae were observed in the intercellular spaces of shoot apical meristem (arrow) tissue. Bar, 100 μ m. (F) Longitudinal section of an *H. sasae*-colonized shoot of *Sasa palmata*. The green color indicates the fungal mycelia of *H. sasae* stained with fluorescein isothiocyanate-labeled wheat germ agglutinin. Plant tissue was stained with safranin. Mycelia were observed on the surface of the shoot apical meristem (arrow). The bright field image and fluorescent images were combined. Bar, 100 μ m.

References

1. Tanaka E. Specific in situ visualization of the pathogenic endophytic fungus *Aciculosporium take*, the cause of witches' broom in bamboo. *Appl Environ Microbiol* 2009; 75:4829-34.
2. Tanaka E, Tanaka C, Gafur A, Tsuda M. *Heteroepichloë*, gen. nov. (Clavicipitaceae; Ascomycotina) on bamboo plants in East Asia. *Mycoscience* 2002; 43:87-93.
3. Cheng Y. Identification of *Heteroepichloë* species on *Brachystachyum densiflorum* by morphology and phytochemistry. *Journal of Beijing Forestry University* 2009; 31:84-90.
4. Tanaka E, Tanaka C. Phylogenetic study of clavicipitaceous fungi using acetaldehyde dehydrogenase gene sequences. *Mycoscience* 2008; 49:115-25.
5. Rykard D, Bacon C, Luttrell E. Host relations of *Myriogenospora atramentosa* and *Balansia epichloë* (Clavicipitaceae). *Phytopathology* 1985; 75:950-6.
6. White JF Jr, Glenn A. A study of two fungal epibionts of grasses: structural features, host relationships and classification in the genus *Myriogenospora* (Clavicipitales). *Am J Bot* 1994; 81:216-23.
7. Tanaka E, Tanaka C, Ishihara A, Kuwahara Y, Tsuda M. Indole-3-acetic acid biosynthesis in *Aciculosporium take*, a causal agent of witches' broom of bamboo. *J Gen Plant Pathol* 2003; 69:1-6.
8. Christensen MJ, Bennett RJ, Ansari HA, Koga H, Johnson RD, Bryan GT, et al. *Epichloë* endophytes grow by intercalary hyphal extension in elongating grass leaves. *Fungal Genet Biol* 2008; 45:84-93.
9. Mowry R, Winkler C. The coloration of acidic carbohydrates of bacteria and fungi in tissue sections with special reference to capsules of *Cryptococcus neoformans*, pneumococci and staphylococci. *Am J Pathol* 1956; 32:628-9.